

Physiology 426
Midterm Paper

**SPECULATIONS ON THE EVOLUTION OF ION CHANNELS IN ELECTRICALLY AND
CHEMICALLY EXCITABLE MEMBRANES**

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NOTE:

Because of the broad scope covered necessarily very superficially by this paper it would be an impossible task within the two-week take-home exam format to include specific references to every sentence. This is especially true for me since I missed two weeks of classes due to medical imperatives and since I am new to the subject and have had to spend most of the time absorbing the recent literature in order to get the big picture. I must assume that comprehensive, formal referencing is not expected; nevertheless I have appended an informal bibliography of the papers and major texts that I consulted in attempting to piece this all together.

To build a plausible hypothesis for the evolutionary relationships between the varieties of ionic channels seen in electrically and chemically excitable membranes, two lines of inquiry must be included: the phylogeny of the biological systems in which the channels are found, and the recent sequencing of cloned channel proteins. Following is a general scenario that I have extrapolated from discussions in the Hille and Shepard textbooks. After offering this scenario I will elaborate on specific channel relationships and discuss conflicting data.

Ca channels may have been the first to play a major role in signaling. Ion channels in general appeared with the first prokaryotes for basic osmotic and metabolic needs. Ca could then have acted as a second messenger for non-channel chemoreceptors that were necessary for responding to nutrients. Bacteria are not known to have action potentials.

In eukaryotes the mitochondria took over ATP generation so the plasma membrane could now utilize a Ca gradient for action-potential signaling and for more extensive second messenger-mediated activities using calmodulin and Ca-dependent protein kinases (and cyclic nucleotides). The K channel delayed rectifier may have been used next to quickly "reset" the action potential and allow quick responsive behavior changes such as the paramecium backing-up response. Thus channel evolution started with Ca and K mechanoreceptor and voltage-gated channels. Already the calcium-activated potassium channel existed, allowing adaptation to constant stimulus, and perhaps even bursting by single cell oscillators.

With the first coelenterate-like metazoans, three developments occurred in parallel: 1) Voltage gated Na channels evolved from voltage gated Ca channels (which fits known channel protein homologies) for intercellular signaling that did not interfere with the extensive intracellular coordinating effects of Ca; 2) gap junctions

appeared and allowed some coordinated behavioral responses by electrical neuroidal conductance such as the jellyfish bell contractions; 3) membrane ligand receptors for nutrients or pheromones become hormone-type receptors for the same chemicals exocytosed by other cells in the organism (as in the cAMP receptor of slime molds) and eventually (after coelenterates) formed pre- and post-synaptic membranes of neurons for quick, one-way signal transfer to local regions. Hormonal and synaptic release used the second messenger effects of the Ca from the original voltage-gated Ca channels.

It is possible that the first ion channels to be activated by neurotransmitters were done so indirectly, by way of the elaborate second-messenger system that was already in place, with the non-channel receptors being derived from the many chemotactic or hormonal receptors that already existed. Later, genetic recombination of the exons of hormone receptor genes that constituted the ligand-binding domains may have incorporated these domains directly into the channel proteins. However, these first ligand-gated channels would probably not have been derived from the same voltage-gated channels already existing; sequence comparisons indicate that they would have diverged at an earlier time, or that they even represent an entirely new line of evolution already existing in protists, perhaps from the mechanoreceptors.

Gap junctions seem to have arisen somewhat independently of the metazoan voltage- and ligand-gated channels, and have maintained a separate line of evolution based largely on facilitating diffusion of morphogenetic gradients. The fact that gap junctions can be gated by intracellular free Ca ions, membrane voltage, calmodulin, and phosphorylation suggests at first that they may therefore have evolved from the protist ion channels. However, while the voltage- and ligand-gated channels employ a "swinging" gate, the connexons of all gap junctions employ

a twisting of the whole unit that brings together the subunits of one end. Secondly, the six 32-kilodalton connexins of a connexon show little homology with the four subunits of voltage- and ligand-gated channels, discussed below, and their channel passes much larger molecules.

Interneurons first appeared in nematodes. The ability to compute with analog signals required accurate translation of the postsynaptic amplitude summations into frequency modulation that is in direct proportion. The appearance of the transient (A current) K channel at this point would have been highly selected for, in order to control the hyperpolarization of the action potential in such a way as to allow this precise encoding.

Finally, further second-messenger potassium-channel modulation allowed habituation and sensitization to begin in nematodes (recently shown by Rankin at UBC), and heterosynaptic facilitation in molluscs, to be topped off by the evolution of the glutamate channel to allow longterm potentiation in mammals.

The Na and Ca channels are both composed of four membrane-spanning subunits, each subunit having six hydrophobic alpha-helix domains, one of which is probably the voltage sensor. The four subunits correspond to four motifs of a single polypeptide. The Na channel sequence is highly conserved across phyla, attesting to its essential, dependable function in action potential generation. It is 60% homologous with the Ca channel that has been cloned from vertebrate muscle (though there is a whole family of Ca channels), and both of them pass the other ion to some degree. The transient potassium channel is probably also four such subunits. Cloning and expression of the *Shaker* locus shows 50 % conservation of the six hydrophobic domains and of the voltage sensor, but it is transcribed from a single gene, from which exons are alternatively spliced to produce variations on the single subunit. Apparently vertebrate transient K channels are homologous. The Ca

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channel may thus have evolved by gene duplication of the transient K channel gene. In fact, paramecium do have the transient K channel, which contradicts my reasoning above for the evolution of the channel. It may be, however, that the new encoding function of the channel took precedence in metazoans.

Sequencing of the nicotinic acetylcholine receptor (nAChR) excitatory Na channel and of the inhibitory glycine and GABA receptor Cl channels shows a similar four-subunit structure, each subunit with only four hydrophobic domains. The two ligand-gated Cl channels show 50% homology, and they in turn are 25% homologous with the nAChR, which fits well with the hypothesized ancestral nature of the nAChR as an effector of muscle responses. However, it could also mean that the difference between a chloride and a cation channel is significant, and the two channel types may have arisen at closer to the same time.

Inhibition by GABA is found in synergistic muscle reflexes in crustaceans as well as in neocortical interneurons, so the channel may have arisen before the split into deuterostome and protostome superphyla. Although inhibition may not have arisen until interneurons arrived on the scene, Cl channels of some sort would have been there from early on. Since they are not found in paramecium, the chloride channels for plant action potentials may have evolved independently. As I have argued previously, ligand recognition-sites on channels probably came later, or were "swapped" from non channel ligand receptors. The fact that GABA is always inhibitory even for GABA-B non-channel receptors and for axo-axonic synapses supports the idea that ligand-gated channels arose from hormone-like systems that had coordinated, consistent behavioral effects.

Because glycine is a neurotransmitter only in vertebrates, and restricted to brainstem and spinal cord, I suggest that it is a recent offshoot of the GABA-A channel,

perhaps then allowing the development of the GABARIN (benzodiazapine recognition) system.

Not only is there little homology between ligand-gated channels and voltage-gated channels, but each subunit of a ligand-gated channel is expressed separately by different genes. Perhaps the degree of subunit "genetic separateness" of the different channel types reflects their relative selection pressures for conservation or innovation -- from one DNA unit (potassium A), to a chain of DNA units (Na, Ca), to separate DNA units (ligand-gated).

As with the ligand-gated channels, the ions that comprise the currents of voltage-gated channels may not be the most important determinants of evolutionary relationships. The transient K-current channel has fair homology with the Na and Ca channels, and it has similar inactivation and I-V characteristics. On the other hand, the delayed rectifier K channel may turn out to be on a separate line of evolution because of slow inactivation and very different I-V curve. It may be expressed by a very different gene with its own regulation of RNA splicing. Similarly, the widespread calcium-activated K channel appears not to be simply a voltage gated K channel that is modulated by calcium -- instead it is the binding of the Ca ion itself that is voltage dependent; the channel itself is voltage independent. These three different K channels all exist in paramecia and may therefore be a result of a major "punctuated equilibrium" in phylogeny. Even the anomalous (inward) rectifier potassium current is found in paramecia. It is unlikely that this channel evolved more recently in paramecia by convergent evolution with metazoans, so all the major potassium channels seem to have arisen in protists and have been strictly conserved in function.

Calcium T-, N-, B-, L-, or whatever-type channels: Invertebrate calcium channels are different from the T/N/L categories for vertebrates. It appears that secretion can

By any of the channels. And

be controlled by any or all of the calcium channels. And from my reading I could not find specific reference to types of channels that generate action potentials. In paramecia, a large part of the calcium spike inactivation is due to calcium-dependent Ca-channel inactivation. Thus the channels responsible for the cardiac spike and for molluscan neuron spikes may have arisen from these original calcium channels. However, the calcium channel that was recently cloned from skeletal muscle and shown to be homologous with the Na channel appears to be the L-type because of its sensitivity to dihydropyridine. It does not inactivate or show calcium-dependence, so its evolutionary relationship to the paramecium calcium channels may be more distant, which would alter the idea that the Na channel evolved from the calcium channel. The fast-inactivating T-type channel may have arisen more recently for boosting postsynaptic summation in long dendrites, such as in pyramidal cells, by producing low threshold action potentials. The slower B-type channel may be responsible for neuroendocrine vesicle release, but, again, may be different from the paramecium channel, so the line of evolution of calcium channels from action potential to hormone release to neurotransmitter release cannot yet be determined. It appears that calcium channels have diverged a lot, in contrast to the conservation of stereotyped categories of potassium channels, which would be consistent with the widespread, flexible use of calcium intracellularly and as a neuromodulator.

Calcium-activated non-specific cation (CAN) channels appear to be another distinct category of channels found in vertebrates and invertebrates, responsible for a variety of effects, and apparently all descended from early eukaryotes, since paramecia have a calcium-activated current carried solely by sodium.

An interesting observations is that the single spike (in paramecium) constituted the mechanism for the first

behavioral responses. Secondly, differential ion channel type distribution on the same nerves or tissue (jellyfish) constituted a means of producing a behaviorally different response to different stimuli. From the first eukaryotes, channels were modulated by Ca-calmodulin and cyclic nucleotides, implying that extremely complex neuromodulation at the single-cell level may have preceded complexity at the circuit level, rather than have evolved as a much later refinement on neuronal network processing, as I myself had thought. Neuromodulation preceded neurotransmitter systems.

Not only that, but the differential alternative splicing of the transient K channel gene transcript implies that even more subtle variations on one ion-channel type may be expressed during development, or at different locations or times in the same organism. The same kind of variation exists between species for the separately transcribed genes of the nAChR subunits, with corresponding variations in conductances. The differences between potassium A channel subunits, however, may have little to do with different channel properties per se, but rather with different phosphorylation sites for second messengers, or different recognition sites for their different tissue locations. Now that cDNA from Shaker has been used to isolate a mouse potassium A channel subunit, it can be seen that the peptide sequence is extremely highly conserved over 600 million years in spite of the fact that the nucleotide sequences are not homologous, having varied greatly within the degeneracy of the code. This must reflect strong selection pressure to maintain the function of the potassium A channel. Whether the degree of subtle variation shown by alternative splicing by Shaker existed in early metazoans or even protozoans is not known.

The kainate-, quisqualate-, and NMDA-type glutamate channels seem to be the only well-known and widespread excitable ion channels about which the structure is unknown. The most recent hypothesis is that the various

pharmacologically distinct types represent different recognition sites attached to the same channel, where each ligand produces a different main conductance state. The NMDA conductance state is also voltage dependent by a mechanism totally different than the other voltage-gated channels -- blockage by a Mg ion. Plus it permits Ca entry as well as Na; plus it may be allosterically potentiated by glycine. All of these aspects of the NMDA receptor, along with the benzodiazapine receptor linked to the GABA-receptor channel, seem to me to represent more recent evolutionary innovations in vertebrates. This would be in keeping with the putative mammalian phenomena of longterm potentiation, perhaps the pinnacle of evolution of neuromodulation, in which both the quisqualic and NMDA receptors are needed, and in which various forms of postsynaptic intracellular second messenger systems produce both post synaptic and retrograde presynaptic longlasting changes.

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